学 位 論 文 の 要 旨

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学　位　論　文　名　Architectural Roles of Multiple Chromatin Insulators at the Human Apolipoprotein Gene Cluster

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論　文　内　容　の　要　旨

INTRODUCTION

Tissue type or developmental stage-specific expression of multiple genes in cluster on mammalian genome may require long-range regulatory elements and higher-order chromatin structure such as the chromosomal domains. In comparison to control of individual genes, chromatin-based mechanisms must have critical roles in regulating the gene clusters which have arisen by tandem duplication events. Chromatin insulators are boundary elements that partition the genome into the chromosomal domains, through their ability to block interactions between enhancers and promoters when positioned between them (enhancer-blocking activity) and/or their ability to block repressive chromatin effects on the flanking regions (barrier activity). The CCCTC-binding factor (CTCF) is known to bind insulators and exhibits the enhancer-blocking function. Genome-wide analyses have then revealed the distribution of putative CTCF-binding sites and their consensus sequences. More recently, using chromatin immunoprecipitation (ChIP)-on-chip studies, we and others have further identified approximately 14,000 CTCF-binding sites on the human genome, which are frequently enriched with the cohesin complexes that mediate sister-chromatid cohesion in mitosis and gene regulation in postmitotic cells. However, the competence of CTCF/cohesin-binding sites for insulation, the functional relationship of CTCF and cohesins, and the implications of insulators in regulating gene clusters are not understood.

The risk of developing dyslipidemia and cardiovascular diseases is increased by high levels of circulating triglycerides in blood, which are often associated with genetic variations in the *apolipoprotein* (*APO*) genes. The *APOA1/C3/A4/A5* gene cluster on human chromosome 11q23.3, is dominantly expressed in liver and intestine, and these genes are crucial for the metabolism and redistribution of lipoproteins and lipids. APOA1, APOA4 and APOA5 are the major constituents of high density lipoprotein (HDL), and the plasma levels of these proteins are negatively correlated with the development of atherosclerotic diseases. In contrast, APOC3 contributes to the formation of very low density lipoprotein (VLDL) and much lower amounts of HDL, thereby suggesting that expression of the *APO* genes need to be appropriately regulated. Furthermore, several single nucleotide polymorphisms (SNPs) within the *APOA1/C3/A4/A5* cluster in human populations are strongly linked to sporadic dyslipidemia and familial combined hyperlipidemia, as well as increased susceptibility to atherosclerosis. Despite the pathophysiological significance of the apolipoproteins, the epigenetic control of the *APOA1/C3/A4/A5* gene locus is largely unknown. The aim of this study is to investigate the role of CTCF and cohesins in the *APOA1/C3/A4/A5* gene cluster.

MATERIALS AND METHODS

To test the hypothesis that chromatin insulation may regulate higher-order control of the gene cluster regions, using ChIP-on-chip tiling microarray analyses, we characterized the potential CTCF/cohesin-mediated insulators in the *APOA1/C3/A4/A5* gene locus. To investigate the role of insulators in the *APO* locus, we utilized RNA interference-mediated knockdown of CTCF and RAD21 in hepatic Hep3B cells. We then checked the expression levels in the *APO* gene region, using the quantitative RT-PCR analysis. Since depletion of CTCF or RAD21 particularly affected the *APOA1/C3* genes, we also checked the existence of HNF4α in the *APO* gene promoters and the *C3* enhancer. We further performed a chromosome conformation capture (3C) analysis to clarify the long-range effects of the insulators on the *APOA1/C3/A4/A5* region, in combination with knockdown of CTCF or RAD21. The relative crosslinking frequency between the reference fragment and other individual fragments was determined by quantitative PCR measurement of three different samples from control and knockdown Hep3B cells (control K.D., CTCF K.D., and RAD21 K.D.). 3C-quantitative PCR data were normalized toward a

loading control, using internal primers located in the *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene, in order to normalize the amount of template DNAs. Statistical analysis was performed by Student's *t* test using more than three independent experiments.

RESULTS AND DISCUSSION

Long-range regulatory elements and higher-order chromatin structure coordinate the expression of multiple genes in cluster, and CTCF/cohesin-mediated chromatin insulator may be a key in this regulation. The human *apolipoprotein* (*APO*) *A1/C3/A4/A5* gene region, whose alterations increase the risk of dyslipidemia and atherosclerosis, is partitioned at least by three CTCF-enriched sites and three cohesin protein RAD21-enriched sites (two overlap with the CTCF sites), resulting in formation of two transcribed chromatin loops by interactions between insulators. The *C3* enhancer and *APOC3/A4/A5* promoters reside in the same loop, where the *APOC3/A4* promoters are pointed towards the *C3* enhancer, while *APOA1* promoter is present in the different loop. The depletion of either CTCF or RAD21 disrupts the chromatin loop structure, together with significant changes in the *APO* expression and the localization of transcription factor HNF4α and transcriptionally active form of RNA polymerase II at the *APO* promoters. Thus, CTCF/cohesin-mediated insulators maintain the chromatin loop formation and the localization of transcriptional apparatus at the promoters, suggesting an essential role of chromatin insulation in controlling expression of clustered genes.

CONCLUSION

CTCF/cohesin-mediated insulators are required for maintaining the overall structure of the *APO* gene cluster.